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SUPPLEMENTARY REMARKS ON THE MICRO-DETERMINATION OF GRAPE SUGAR

[Ergänzende Bemerkungen über die Mikrobestimmung des Traubenzuckers]

by

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from: Biochem. Z. [Biochemical Journal] 87: 264-72 (1918)

(Received 8 November 1917)

The micromethod for sugar determination in its present form has two sources of error which require special caution during execution: 1. Air oxidation, and 2. the intensity of the boil. In the foregoing paper one of us gave precautions by which the two sources of error can be shut out. With regard to the second source of error, the intensity of the boil, a gas pressure regulating mechanism was recommended. This regulator had already functioned satisfactorily for about one year, when finally the progressive deterioration of the gas (uneven mixing with

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wood gas) rendered the entire apparatus nugatory. In order not to have to give up sugar determination entirely, the problem was further worked on, until it was finally possible to overcome decisively the entire source of error.

In concordance with Ege[1] we first tried extending the boiling period in the hope that irregularities could be compensated in this manner. The results, however, were not satisfactory, as the following experimental series demonstrates.

0.20mg sugar used in all cases. Boiling time 1 min. = 0.11 mg sugar found. 2 min. = 0.215mg, 3 min. = 0,29mg, 4 min. = 0.34mg, 5 min. = 0.38mg and 6 min. = 0.40mg. In the last experiments the solution had cooked down to a salt brine. According to Ege, this difficulty can be eliminated by the addition of 5cc water to the original solution. There remains, however, the disadvantage that the procedure takes a long time. Additionally, Ege himself found that the intensity of the boil asserts itself after a boiling time of 7 minutes.

If we reflect why this variable intensity of the boil should be of such importance, it is apparent that this factor cannot directly alter the temperature of the alkaline salt solution. A 20% KCl solution boils at approximately 102° irrespective of whether one uses a high flame or a low one. However, in the former case more water evaporates than in the latter. The consequence is that the salt solution becomes more concentrated, and the temperature rises. Likewise the solution's alkalescence rises. Both factors occasion an increased reduction effect for sugar.

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[1] Ege, Ugeskrift for laeger No. 4 (1917).

if, therefore, one could see to it that the volume of the fluid remains unchanged during boiling, or at least is not decreased, it would seem that any variation in the intensity of the boil would have much less effect. This requirement is best fulfilled by introducing indirect heat via boiling water vapor, as in the micro-Kjeldahl Method after Bang. This provides that the alkaline salt solution is raised to the boiling point, and gradually, as the amount of condensation water increases, the boiling point sinks, while alkalescence is lessened. Finally, one need not worry about any retardation of the boiling point or influences from the irregularities in the glass. One need only register the time at which the vater vapor is introduced into the flask. After the completion of heating, 2cc of 20% sulphuric acid is added and iodate consumption is determined by titration with n/300 thiosulfate solution (see previous paper).

Thanks to this experimental arrangement it developed that we obtained good and congruent values, even when the amount of water vapor varied quite considerably. This was achieved by heating the boiling flask more or less quickly. The water vapor achieved was introduced into the flask with the sugar and alkaline salt solution (see Fig. 2). Thus, with the use of 0.20mg of sugar at high heat over 5 minutes, 1.39cc and 1.39cc of thiosulfate solution were consumed. During very fast heating, 1.41cc and 1.42cc thiosulfate solution. With moderately fast (normal) heating, 1.40cc and 1.38cc thiosulfate solution. Only with very slow heat did we get 1.84cc and 1.94cc thiosulfate solution.

Although these values, too, were satisfactory, it was nevertheless apparent that an extended boiling time produced a greater reduction:

With a boiling time of 6 minutes, 1.31cc and 1.26cc thiosulfate, after 7 min. 1.15cc and 1.13cc; after 8 min. 0.98cc and 0.98cc and after 9 min. 0.96cc and 0.97cc thiosulfate solution. Only after 8 minutes of boiling time was the reduction finished. But such a long boiling time is simply not practical, and therefore experiments were begun with the goal of shortening the reduction time. It was conceivable a priori that this could be achieved by greater alkalescence, since the literature contains data according to which greater alkalescence leads to more vigorous reduction. Our experiments, however, yielded precisely the opposite results. Besides the 2cc of carbonated iodate solution we added in some experiments 2cc of 10% potash lye-- and, additionally, in some experiments Seignette salt. With the use of 0.20mg sugar we arrived after a boiling time of 3 min. at 2.19cc and 2.19cc thiosulfate solution; after 4 min. at 2.18cc and 2.20cc and after 5 min. 2.19 and 2.19cc thiosulfate. From this it is evident, first, that only a little copper oxide is reduced (the quantity is equivalent to 0.05mg sugar) and secondly, that the reduction is finished after only a short time. One can imagine that two processes are taking their course here side by side. In part the sugar is oxidized by the copper oxide, but in part it is destroyed by the lye in such a way that the products cannot be further oxidized by the copper oxide. Under this assumption, an increase in copper could promote a greater reduction; as was in fact the case. By the addition of 0.2cc of 3% copper sulfate solution and 2cc of potash lye (in addition to 2cc of carbonate solution and 13cc salt solution) we arrived at 1.98cc thiosulfate for 0.10mg (sic) sugar, 1.82cc for 0.2cc and 1.45cc for 0.3cc, all at a boiling time of 5 minutes.

Out of these experiences there arose the task of regulating the the reciprocal relationship between copper and alkali in such a way that the best conditions for sugar reduction reign. First we tried exchanging the carbonate for potash lye (up to 0.5%) and Seignette salt. Using 0.20mg of sugar we achieved practically no reduction at all with 2cc of alkaline iodate solution. Only after the addition of K_2CO_3 to this solution was a reduction effect achieved: After the addition of 0.50cc of 10% K_2CO_3 solution, 1.62cc thiosulfate solution was consumed, after the addition of 1.00cc -- 1.46cc; after the addition of 1.50cc -- 1.61cc and after the addition of 2cc -- 1.69cc, all at a boiling time of 3 minutes, with 0.20mg sugar. The optimum, as is evident, lies at 1.00cc of 10% K_2CO_3 solutionto 2cc of alkaline iodate solution.

What was more important, however: The reduction effect amid variations of the boiling time from 2 to 6 minutes was completely unchanged. Findings: boiling time 1 min. = 1.98cc; 2 min. = 1.60cc, 2 min. 30 sec. = 1.58cc, 3 min. = 1.59cc, 4 min. = 1.61cc, 5 min. = 1.60cc and 6 min. = 1.60cc. In all these experiments 1.50cc of carbonate solution was added.

However satisfactory these results seemed, further experiments nevertheless showed differences in the reduction effects of various sugar amounts. Using 0.20mg sugar, the iodine consumption per 0.01mg sugar equaled 0.021 cc n/100 iodine; using 0.4mg, though it was 0.025 cc n/100 iodine. The difference is to be sure small, and furthermore, given the use of an empirical reduction table, it is inconsequential. But we declined to work out such a table, since it appeared

that a change in the alkalescence caused a quite regular reduction. To this came the additional fact that the reduction per 0.01mg sugar was even greater, namely 0.028cc n/100 iodine solution (or 0.056cc n/200 iodine). The reduction is some 3/2 greater than with the original procedure.

The composition of the alkaline iodate solution is accordingly: 75g K_2CO_3, 20g Seignette salt and 0.3567g KIO_3 (= n/100 iodate) dissolved in 1 liter water[1]. Both the lye and (on the other side) the bicarbonate are removed. On the other hand, the more vigorous reduction uses more iodate than originally. Accordingly we also added somewhat more copper sulfate to the salt solution, namely 800mg $CuSO_4 + 5H_2O$ instead of the previous 500mg -- all to 2 liters salt solution.

The results which we obtained with this reduction fluid are compiled in the following table (see also Fig. 1). In all cases 2cc iodate solution + 13cc salt solution were used.

Kochzeit[a]	mg Zucker gefunden für:[c]				
	0,10 mg	0,20 mg	0,30 mg	0,40 mg	0,50 mg
1 Minute[b]	0,054	0,104	0,154	0,200	0,240
2 Minuten	0,086	0,168	0,246	0,346	0,432
2½ "	—	0,189	—	—	—
3 "	0,100	0,200	0,289	0,382	0,482
4 "	0,104	0,200	0,300	0,404	0,490
5 "	0,100	0,200	0,304	0,404	0,490

In three blind experiments, 0.03, 0.03 and 0.05cc iodine were found.

Key: a. Boiling time
b. Minutes
c. mg Sugar found for:

[1] The iodine amount can probably be decreased somewhat, e.g. 0.2854g = n/125, since more than 0.5mg sugar occurs in any case only very infrequently.

The calculation is very simply (1.97 ÷ a) : 2.8 equal to the value sought for sugar. 1.97 corresponds to the 2.00 n/100 iodine solution (as iodate) present, less the iodine consumption of the blind analysis. This consumption of iodine is calculated for very pure reagents. The 2.8 factor corresponds to the reduction for 0.01mg sugar which, as can be seen, is quite constant everywhere.

It is evident from the table that the main part of the reduction is completed during the first two minutes. In the third minute the reduction is but slight, and after 3 minutes, for lesser sugar values of up to 0.20g, the reduction is complete. In the presence of more sugar a slight reduction still takes place during the third minute. But since these sugar values would occur but seldom, one can undoubtedly prescribe a boiling time of 3 minutes. It is better, however, to extend this boiling period to 4 minutes. In these circumstances it is of no significance whether the boiling lasts a minute more or less. For this reason it is recommended that a boiling time of 4 minutes always be used.

According to the above findings it is not surprising that the <u>intensity of the boil</u> has become quite inconsequential. The following experimental series can inform about this. In all cases 0.20mg sugar were used.

1. With open gascock without wire net, 0.208mg found,
2. " " " with " " , 0.200mg " ,
3. " 72/90 " " " " " , 0.200mg " ,
4. " 54/90 " " " " " , 0.200mg " ,
5. " 38/90 " " " " " , 0.160mg " ,

Except for the extremes one therefore gets quite correct values with a wide variety of boiling intensities.

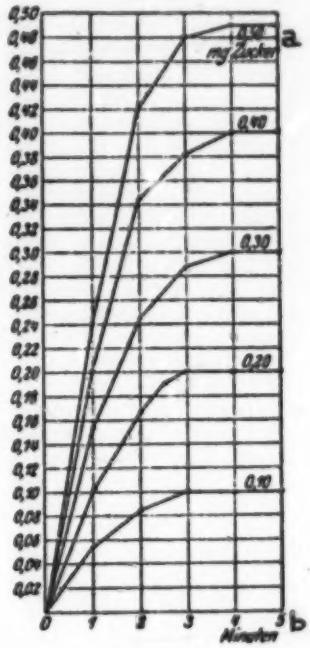

Fig. 1.

Key: a. Sugar b. Minutes

Thanks to the modifications described in the last two papers, the two earlier sources of error attached to the procedure have been completely eliminated. In the process the method has become simpler and also more exact. In fact, in its present form it is a precision procedure hardly second to even the best macromethods. Finally, as evidence for the precision of the procedure, we should like to communicate the following experiments. They were carried out in such a way that the sugar solutions were measured out by one of us and determined by the other. We should like to remark explicitly that all experiments are

recorded.

Zucker berechnet[a] mg	Gefunden[b] mg	Differenz[c] mg
0,04	0,043	+0,003
0,05	0,047	−0,003
0,06	0,061	+0,001
0,13	0,128	−0,002
0,14	0,143	+0,003
0,15	0,154	+0,004
0,16	0,169	+0,009
0,17	0,168	−0,002
0,18	0,182	+0,002
0,25	0,243	−0,007
0,27	0,275	+0,005
0,35	0,357	+0,007

Key: a. Sugar calculated
b. Found
c. Difference
(all commas = .)

The results were obtained with the use of n/100 thiosulfate solution. Perhaps it would be possible to reduce the experimental error even further by means of n/200 thiosulfate solution[1].

Procedure

13cc of salt solution containing sugar (after extraction of sanguineous paper) containing per 2 liters 400g KCl, 3.0g uranyl acetate, 1.5cc of 25% hydrochloric acid and 800mg copper sulfate, together with 2cc of iodate solution containing per 1 liter 15g[2] K_2CO_3, 20g Seignette salt and 0.3567g potassium iodate, are introduced into a narrow-necked 125cc Erlenmeyer flask. Previously the

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[1] It should be remarked that the method has also proved its value for blood sugar determination.

[2] [Footnote subsequently added:] Should be: 75g K_2CO_3. See "Berichtigung" following p. 424, V. 87, 1918.

boiling flask (a 500cc Jena glass standing flask) is filled 2/3 full with distilled water and a few boiling stones (air-dried clay fragments), and heated to boiling on a wire net over a Bunsen burner. When the distillation tube is warmed through (while the wider glass tube is shut with a pinch-cock), the Erlenmeyer flask is placed below. The tip of the distillation tube should stand as deep in the fluid as possible. The ball-shaped widening in the distillation tube is meant to prevent splashing. (The distillation tube is made from a 20cc pipette). The stopper is cork, rubber must not be used. The start of heating is noted by means of an hourglass (a 4-minute hourglass is better than stopwatches, which malfunction after a while; it is also much cheaper). After 4 minutes have passed, 2cc of 20% sulfuric acid is introduced into the solution from a pipette and the side tube is simultaneously opened. Afterwards the boiling flask is raised and the distillation tube rinsed with a wash bottle. After standing, the flask is cooled for 5 minutes[1] under the water faucet, one adds ca. 25cc water, 2 drops of 10% potassium iodide solution and 2 to 3 drops of 1% starch solution, and titrates until the liquid turns colorless. After standing for some time the blue color returns. If the return of blue is observed during the first 5 minutes, titration must be resumed. Usually ½ to 1 drop is now enough for the change. From the formula 1.97 ÷ of the consumed thiosulfate solution (n/100) divided by 2.8, the sugar value is obtained.

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[1] In two experiments the fluid was cooled immediately after heating. Instead of 0.20mg sugar, 0.182mg and 0.180mg were found.

Instead of the above solutions one can add the iodate -- 0.2cc n/100 KIO_3 [1] -- by itself and likewise 2 drops of a 10% $CuSO_4$ solution.

Only absolutely pure potassium iodate should be used. Occasionally uranyl acetate also contains iodine bonding substances and must then be purified -- preferably through conversion to chloride by heating and dessication with hydrochloric acid.

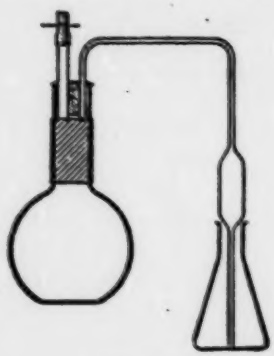

Fig. 2.

[1] [Footnote subsequently added:] Should be: n/10 KIO_3. See "Berichtigung" following p. 424, V. 87 (1918).